J. Exp. Biol. (1970), **52**, 291–298
With 3 text-figures
Printed in Great Britain

WATER BALANCE IN THE SALMON EGG

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(*Received* 9 *September* 1969)

INTRODUCTION

It seems to be a general rule that the tonicity of eggs is closely similar to that of the body fluids of the maternal organism. This circumstance entails that, particularly in higher animals, a substantial tonicity difference may exist between the egg and the medium in which it develops. Confining ourselves to the teleosts, it is a fact that sea water is hypertonic to the eggs of many marine fishes and fresh water is strongly hypotonic to the interior of all eggs developing in this medium. It may therefore be anticipated that, unless they possess some means of osmoregulation, the eggs must undergo substantial changes in volume upon shedding. Slight adjustments may actually be observed, often in association with fertilization, but these soon come to a halt, and subsequently the volume remains constant for long periods in spite of a demonstrable difference in tonicity between the egg and its environment. This obviously must imply that eggs and embryos are endowed with a mechanism by which their volume is regulated.

Various suggestions have been advanced to account for this volume regulation. Thus it has been envisaged that they may be able to perform osmotic work (cf. Straub, 1929; Tuft, 1962). It is well established that specialized cells may carry out this kind of activity, but to our knowledge no convincing evidence has ever demonstrated its presence in eggs and embryos.

Another possibility is that the plasma membrane is impermeable to water. Both Loeb (1912), working with the eggs of *Fundulus*, which are hypertonic to their medium (sea water), and Gray (1932), working with eggs of *Salmo* which develop in fresh water, have advanced this opinion. Their view was based on the fact that no net exchange (loss or gain) of water occurred when the eggs were placed in solutions of varying tonicities. It must be emphasized, however, that this method cannot decide whether the constancy of volume is due to impermeability of the plasma membrane or to some other mechanism which opposes swelling or contraction of the egg.

This question might be settled by using isotopic water which permits the demonstration of exchange even when the total volume remains constant. In their pioneer work Krogh & Ussing (1937) found that there is virtually no exchange of D_2O in salmon eggs, and this result has been confirmed by Prescott (1955). Although the impermeability of the plasma membrane to a small uncharged molecule like that of water appears to be physiologically improbable, it has won general acceptance. Its universal validity is suggested in the following quotation: 'eggs of marine and fresh-water fish and probably of most fresh-water animals become virtually impermeable to water' (Prosser & Brown, 1961, p. 27).

The isotope-exchange method has not been used extensively, but it is remarkable that those authors who claim that eggs are impermeable have generally worked with large eggs; determinations of the rate of water exchange in smaller eggs do not give particularly low values (cf. Prescott & Zeuthen, 1953; Loeffler & Løvtrup, 1969), although the problem of osmoregulation must increase in proportion to the surface: volume ratio. These findings appear to raise doubt as to the validity of the generalization cited above, and suggest that regulation of the volume is not always achieved by sealing off the water exchange through the plasma membrane.

As regards other means by which the volume may be regulated we may consider amphibian eggs developing in fresh water. It has been demonstrated repeatedly that these eggs are freely permeable to water (cf. Prescott & Zeuthen, 1953; Løvtrup, 1960; Haglund & Loeffler, 1968). Yet they do not swell very much after shedding, and it therefore is necessary to invoke some other principle to account for volume regulation in this case. The most obvious suggestion is that the osmotic forces are opposed by the mechanical tension known to exist at the surface of the egg. It has been found that most of this tension is exerted by the vitelline membrane in body-cavity eggs, but the determinations made so far do not make it possible to calculate whether the tension is high enough to balance the osmotic pressure difference between the egg and the environment (cf. Berntsson, Haglund & Løvtrup, 1965).

In spite of all the work done on this problem, we have become convinced that no solution has yet been obtained. This has led us to ask once more the by now classical question: Are salmon eggs impermeable to water? If not, is the permeability so low that this factor alone suffices to account for the volume regulation observed?

MATERIAL AND METHODS

Fertilized eggs of *Salmo salar* were obtained from the local fish hatchery. They were placed in 7·5% Ringer, and stored in a cold room (4° C.).

The exchange of water was followed by determinations of reduced weight (*RW*) according to the method of Pigon & Zeuthen (1951) and Løvtrup & Pigon (1951). The *RW* determinations were made on the automatic electro-magnetic diver-balance (Larsson & Løvtrup, 1966). When the rate of water exchange is fast, it is possible to record most of the course of the reaction. Since the process follows approximately that of a first-order reaction, the rate constant is independent of any absolute units and calibration of the balance is therefore unnecessary. When the exchange reaction extends over many hours or days, it is inconvenient to leave the object on the balance. For one thing the instrument is taken out of service, and only one experiment can be performed at a time; and in any case it would be necessary to take off the object from time to time in order to check the zero point. Under these circumstances it is much more expedient to make determinations of the *RW* at convenient intervals. Provided the points thus obtained describe a first-order reaction, it would not be necessary to know any absolute values. However, when experiments last for several days, it is comforting to possess some means of checking the results. We have therefore calibrated the diver balance by means of platinum standards from time to time.

In order to accommodate the large salmon eggs a special diver had to be constructed (Fig. 1).

The rate of water exchange between a cell and its surrounding depends not only upon the rate at which the water passes through the plasma membrane, but also upon the rates of diffusion inside and outside the cell. The latter factor is generally neglected, but the diffusion coefficient (D) for water in cytoplasm must be known before the permeability coefficient (E) can be calculated (Løvtrup, 1963). In order to estimate the former, it is necessary to have available an egg deprived of its plasma membrane, i.e. a

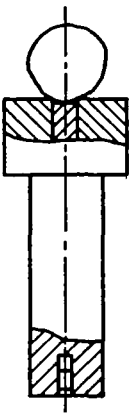

Fig. 1. Diver loaded with a salmon egg. The body of the diver is made of polyethylene, the collar of polyvinyl chloride. Enclosed in the body are a piece of magnetic material (Koerzit T) adjusted to give a proper sensitivity, and a piece of platinum adjusted to give a proper buoyancy.

cytolysed egg. We have made several determinations of D on spontaneously cytolysed eggs, but such eggs are not always available when required, and it may therefore be convenient to provoke cytolysis at will. It has turned out that treatment with 70% alcohol serves this purpose well (Haglund & Loeffler, 1969), the values of D determined on these eggs being indistinguishable from those obtained on cytolysed eggs.

In order to calculate the coefficients, a parameter relating to size of the egg must be known. The equations employed presume a spherical object with radius R, but in fact the salmon egg is a prolate spheroid, the major axis running through the animal and the vegetal poles. The major axis, a, and the minor axis, b, of an egg were determined from several measurements with an ocular screw micrometer. From these values the 'radius' R was calculated as

$$R = \frac{a+2b}{3}.$$

Such determinations were often repeated during an experiment. The value of R thus obtained refers approximately to the volume confined by the chorion, which for cytolysed or alcohol-treated eggs corresponds to the volume partaking in the exchange reaction. The situation is slightly more complicated in normal eggs, because the volume surrounded by the plasma membrane is only a fraction of the total volume, the rest (20–25% as determined by means of the micrometer) representing the perivitelline space. It might be possible, but not easy, to introduce a correction; however, rather than attempt this approach we have accepted that our calculated values of E may be wrong by 10–15%.

The experiments reported here were carried out as follows. A number of glass tubes, each containing an egg in 7·5% Ringer, were placed in the diver-balance water-bath (5·5° C). The exchange reaction was started by transferring the egg to a medium containing 20% D_2O in 7·5% Ringer, the same as the flotation medium of the balance. After the change of medium the egg was quickly transferred to the diver to determine the initial *RW*. Subsequently it was placed in the isotope medium, where the exchange was allowed to continue, interrupted by weighings at convenient intervals. One experiment involved three eggs, each of which was started at a specified time during the first day. By this expedient it was possible to get determinations quite evenly distributed with respect to time, without working through the 24 hours of the day.

The eggs might vary with respect to volume, and thus to the uptake of isotopic water, or change in *RW*. In order to combine the results it was necessary to introduce a correction; this was achieved by determining the total change in *RW* for each egg, and using these values to get correction factors for the smallest and the largest egg, which were used to adjust the corresponding values. The ensuing points were clustered in groups of 3–4 at close intervals along the time axis, and for each of these the mean value was calculated. These values were plotted in a graph, and used to calculate the first-order equation which gave the best fit to the experimental points. The mean of the three individual *R* values was used in the calculations.

RESULTS

The results obtained with alcohol-treated eggs are shown in Fig. 2, those with normal eggs in Fig. 3. It is seen that in both cases there is a phase of very rapid exchange during the first 15–30 min. which is not accounted for by the calculated curves. It is

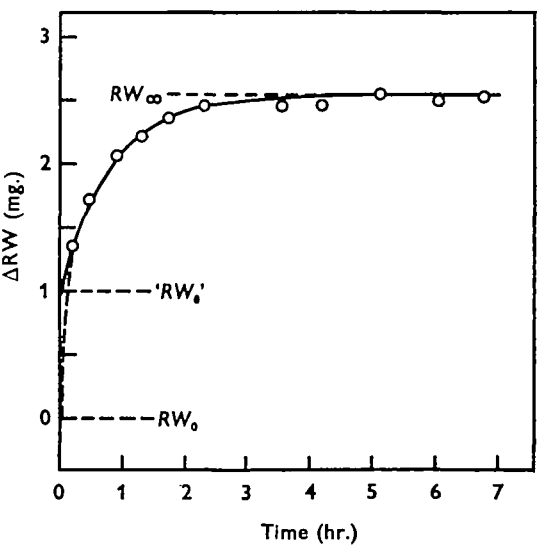

Fig. 2. Water exchange in alcohol-treated salmon eggs. The curve is the first approximation of an infinite series representing the water exchange in a sphere with no diffusion barrier at the surface. RW_∞ and 'RW_0' are the calculated values for $t = \infty$ and $t = 0$. RW_0 is the actually observed zero point. The difference 'RW_0' $- RW_0$ is a mathematical artifact (see text).

probably most easy to explain this phenomenon in the case of Fig. 3. Here the calculated curve intersects the ordinate axis at 'ΔRW_0' = 0·56 mg., whereas the value of ΔRW_∞ corresponding to the curve is 2·74 mg. The part of the water exchange not accounted for by the curve is thus 20·5%, which shows that the rapidly exchanging water must represent that present in the perivitelline space.

In Fig. 2 the corresponding values for ΔRW are 1·00 and 2·56 mg., suggesting that 39% of the total exchange is faster than the rest. At first sight this appears astonishing, since it may be anticipated that in alcohol-treated eggs there is one homogeneous water phase beneath the chorion. It must be pointed out, however, that the equations used

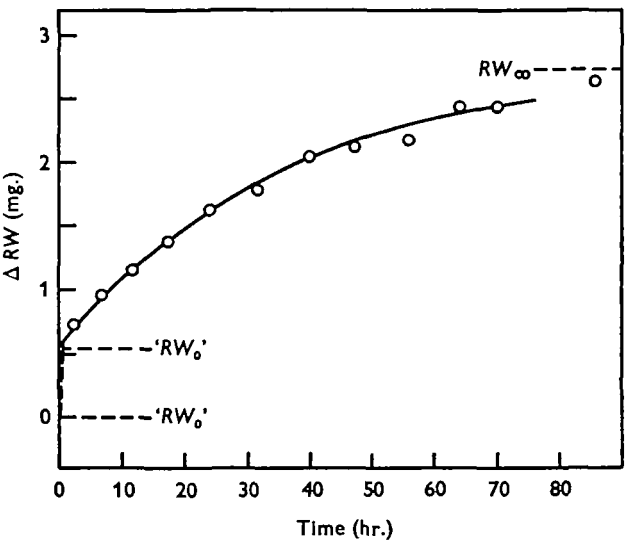

Fig. 3. Water exchange in normal salmon eggs. The curve is the first approximation of an infinite series representing the water exchange in a sphere with a diffusion barrier at the surface. The significance of RW_∞, 'RW_0' and RW_0 are as in Fig. 2. The difference 'RW_0' – RW_0 corresponds approximately to the perivitelline space.

for calculating the curves are the first approximations of those infinite series which theoretically describe the course of the water exchange. It can be shown that the agreement between the infinite series and the first approximation is better the slower the rate, and particularly when a diffusion barrier (plasma membrane) is present at the surface. In the absence of a membrane a difference obtains between the two equations, which in the limiting case gives a value of $\Delta RW_\infty - $'$\Delta RW_0$' that is too low by a factor of $\frac{1}{8}\pi^2$ = 1·64 (for mathematical details, see Løvtrup, 1963). In the present context $\Delta RW_\infty - \Delta RW_0$ should thus be 1·64 × 1·56 = 2·56 mg. The exact agreement is fortuitous, but the calculation nonetheless supports the suggested interpretation. From the curve it is found that the diffusion coefficient D for water in the 'cytoplasm' of the salmon egg is $4{\cdot}1 \times 10^{-6}$ cm.^{2} sec^{-1}, the half-time for the exchange being 33 min.

Using this value, it is possible to calculate the value for the permeability coefficient E which is thus found to be $1{\cdot}0 \times 10^{-6}$ cm. sec.$^{-1}$, the half-time in this case being 23 hr.

DISCUSSION

From the results obtained it must be concluded that salmon eggs are permeable to water, but it has to be admitted that the rate of exchange is exceedingly slow. Before we discuss the consequence of this finding for the problem of volume regulation, we must try to establish, by comparison with results obtained on other species, whether the plasma membrane in the salmon egg is unusually tight.

The work reported here was carried out at 5·5° C., which is in the optimum temperature range for salmonid eggs. Observations at such low temperatures are rare, but fortunately some determinations have been made on amphibian eggs (Haglund & Løvtrup, 1966). It appears that in this case D is about 2×10^{-6} cm.^{2} sec.$^{-1}$, and E about $1\text{–}2 \times 10^{-5}$ cm. sec.$^{-1}$.

The diffusion of water in the salmon egg is thus about twice as fast as in amphibian eggs, but the plasma membrane appears to be 5–10 times less permeable. The membrane is thus unquestionably a tight membrane, but its permeability coefficient lies within one order of magnitude of that found for the amphibian egg, a circumstance which hardly warrants that it be characterized as exceptional.

The very slow rate of exchange, responsible for the repeated failure to demonstrate an uptake of isotopic water, is the outcome of three factors in cooperation, the low temperature, the large size of the egg, and the permeability coefficient of the membrane.

We have so far discussed only reports claiming the impermeability of the salmon egg to water. It is necessary to mention that on two occasions the permeation of tritiated water has been reported (Kalman, 1959; Potts & Rudy, 1969). The former author did not make any quantitative estimations. The latter authors followed the uptake for but a few hours, and thus never obtained a complete exchange curve. Without being able to correct for the influence of D, they arrived at an estimate of $E < 0{\cdot}4 \times 10^{-6}$ cm. sec.$^{-1}$, which differs only by a factor of 2·5 from that determined by us.

Having established that the plasma membrane is permeable to water we may turn to the question of volume regulation. It is of decisive importance to know whether or not the egg is in osmotic equilibrium with the environment. If it is, then we are forced to assume that non-osmotic forces are involved, as in the amphibian egg. If it is not then the egg will simply swell constantly during development.

An approximate estimate of the rate at which swelling takes place may be obtained in the following way:

The amount of water passing through the membrane in one direction per unit of time is

$$\frac{dW}{dt} = E \times A \times a,$$

where E is the permeability coefficient, A the area, and a the activity of the water, which for the time being is taken to be 1. With $R = 3$ mm we have

$dW/dt = 1{\cdot}0 \times 10^{-6}$ cm. sec.$^{-1} \times 4\pi \times 9 \times 10^{-2}$ cm.$^2 \times 3600 \times 24$ sec./day $\approx 0{\cdot}1$ cm.^{3}/day.

The volume of the egg is

$$4\pi \times 27 \times 10^{-3}/3 \approx 0{\cdot}1 \text{ cm.}^3.$$

Thus, if water was flowing in one direction only, an amount of water corresponding to the volume of the egg could pass through the membrane in 24 hr. However, water is passing in both directions, at rates proportional to the activity of the water. It is very difficult to determine this parameter, but in a similar calculation an attempt was formerly made to use the vapour pressures for this purpose (Løvtrup & Pigon, 1951). Assuming that the egg is isotonic with Ringer ($\approx$ 220 mM non-electrolyte) and that the outside medium is 7·5% of this value, we arrive at the following values for the vapour pressure (at room temperature)

Water	17·865 mm. Hg,
Medium	17·860 mm. Hg,
Egg	17·800 mm. Hg.

The activities of water in the two phases are consequently 17·860/17·865 and 17·800/17·865, and the net uptake of water is

$$0{\cdot}1 \times \frac{17{\cdot}860 - 17{\cdot}800}{17{\cdot}865} \approx 1{\cdot}0 \times \frac{1}{300} \text{ cm./day.}$$

In other words, the uptake of water is around 1/300 of the egg volume per day; in 50 days it should thus be around one-sixth, a value which is close to that reported by Potts & Rudy, 1969. These calculations show that there is no reason to presume that the salmon egg ever comes in osmotic equilibrium with its external medium. Owing to special circumstances, the low temperature at which it develops, the large size of the egg and the relatively tight plasma membrane the net uptake of water can be confined within narrow limits.

It must be pointed out that the salmon egg represents an exceptional case, most fish eggs are smaller, and the values of E known so far (zebra fish, Zeuthen & Prescott, 1953; pike, Loeffler & Løvtrup, 1969) are higher than that of the salmon egg, and many eggs also develop at higher temperatures. Under these conditions there is reason to believe that special mechanisms to prevent excessive swelling must be present.

SUMMARY

1. The rate of water exchange in the salmon egg was determined by means of the electromagnetic diver-balance.

2. Salmon eggs treated with alcohol to remove the plasma membrane were used to determine the diffusion coefficient, D, for water in the cytoplasm, and untreated eggs to determine the permeability coefficient, E, for water through the plasma membrane.

3. It was found that D is of the same order as that observed in other eggs, whereas the value of E is somewhat lower, but still within the same order of magnitude.

4. The results do not confirm the classical notion that the salmon egg is impermeable to water, but they show that the exchange is extremely slow, the half time being about 24 hr. In order to explain this result the contribution of egg size and environmental temperature must be taken into account.

5. It is concluded that the actually observed rate of swelling corresponds to that anticipated from the recorded permeability coefficient, and that there is therefore no reason to presume that any kind of osmoregulation takes place.

We gratefully acknowledge the assistance of Mr Börje Engström. The present work was supported by Statens Naturvetenskapliga Forskningsråd (grants 2240-15-7463 and 2240-18-7990 *B*) and by a U.S.P.H.S. Postdoctoral Fellowship from the National Institute of Child Health and Human Development, IF2 HD-30, 937-01.

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